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Isolation and properties of alpha granules and ribosomes from a blue-green alga, Nostoc muscorum

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ISOLATION AND PROPERTIES OF ALPHA-GRANULES AND RIBOSOMES FROM A BLUE-GREEN ALGA, NOSTOC MUSCORUM

by

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INTRODUCTION

The blue-green algae occupy a unique position among many other organisms. They have a procaryotic cellular organization which is similar to that of bacteria. They differ from bacteria, however, in that they are generally obligate photoautotrophs, obtaining their carbon and energy by photosynthetic mechanisms which are similar to those found in higher plants. Because of many similarities between chloroplasts and blue-green algae, study of these relatively simple organisms has given rise to some interesting speculations. It has been suggested (Mereschkowski, 1905; Famintzin, 1907; Ris, 1961; Sagan, 1967) that chloroplasts of higher plants may have originated as independent cells. Presumably these invaders were ingested by other cells and established as intracellular symbionts. Over a great length of time these symbionts became organelles of the host cell, losing their ability to live independently. There is considerable morphological evidence in support of the suggestion.

The biochemical nature of chloroplast components has been studied in recent years. Chloroplast ribosomes have been well-characterized, yet there were no reports on the nature of blue-green algal ribosomes. Since chloroplast ribosomes and cytoplasmic ribosomes differ in their sedimentation properties (Clark et al., 1964), blue-green algal ribosomes were isolated for comparative studies. In the course of this work cytoplasmic fractions were obtained rich in α-granules. These were purified and characterized in the hope of elucidating the similarities and differences between chloroplasts and blue-green algae.

LITERATURE REVIEW

Alpha-Granules

Small granules between the thylakoids in the cells of blue-green algae have long been observed (Niklowitz and Drews, 1957; Ris and Singh, 1961; Menke, 1961; Pankratz and Bowen, 1963; Fuhs, 1963). Several terms were used to describe these granules including granular inclusions (Ris and Singh, 1961), crystalline structures (Menke, 1961) and alpha-granules (Pankratz and Bowen, 1963). These granules were stained heavily by lead hydroxide (Ris and Singh, 1961) and were removed by diastase treatment (Fuhs, 1963; Giesy, 1964; Maugini, 1967).

These granules are apparently present in all blue-green algal cells. They appear fairly uniform in diameter but different in length. The average diameter of these granules range from 25 to 30 nm in Gloeocapsa alpicola (Ris and Singh, 1961), 30 nm in Symploca muscorum (Pankratz and Bowen, 1963) to 50 nm in Oscillatoria amoena (Fuhs, 1963). Echlin (1964) reported the presence of 25 by 40 nm rod-shaped granules in Anacystis montana. Long rod-shaped granules are present in Oscillatoria chalybea (Giesy, 1964) and Oscillatoria rubescens (Jost, 1965) where the average diameter is 35 nm but the length may reach 300 nm or more.

Alpha-granules are present in vegetative cells as well as in young akinetes, but are reportedly absent in mature akinetes. Numerous α-granules appear again during akinete germination (Miller and Lang, 1968). Early biochemical studies indicate that a glycogen-like fraction was present in Oscillatoria princeps (Fedrick, 1951). However, no close correlation

between biochemical and morphological observations has been made.

Ribosomes

Since Tissieres and Watson (1958) first isolated ribosomes from Escherichia coli, numerous studies have been made on ribosomes from both higher organisms and bacteria. One interesting report by Clark et al. (1964) was that Chinese cabbage leaves contain two distinct classes of ribosomes. The cytoplasmic ribosomes sedimented at 83S and the chloroplast ribosomes sedimented at 68S. Further studies of Boardman et al. (1966) revealed that chloroplast ribosomes dissociate reversibly into 50S and 30S subunits, in a way analogous to Escherichia coli ribosomes. There has been no information available, however, on the physical properties of blue-green algal ribosomes.

It is known that chloramphenicol inhibits in vitro protein synthesis in Escherichia coli (Nirenberg and Matthaei, 1961, Nathans and Lipmann, 1961). Vazques (1963, 1964a) reported that this inhibition is correlated with the binding of chloramphenicol to 50S ribosomal subunits of both Escherichia coli and Bacillus megaterium. He further established that chloramphenicol fails to bind ribosomes of resistant bacterial strains or heavy type (80S) ribosomes of higher organisms (Vazques, 1964b). Cycloheximide, on the other hand, inhibits protein synthesis of eucaryotic organisms ranging from yeast (Kerridge, 1958) to mammalian cells (Gorski and Axman, 1964). The mechanism of cycloheximide inhibition is not yet clear, but it has been shown that cycloheximide prevents the transferring of amino acids from aminoacyl-s-RNA into proteins (Siegel

and Sisler, 1963). Some preliminary studies indicated that the growth of certain blue-green algae are inhibited by chloramphenicol (Foter et al., 1953; Galloway and Krauss, 1959). Detailed study on drug sensitivity of blue-green algal ribosomes is lacking.

MATERIALS AND METHODS

Culture Preparations

A unialgal culture of Nostoc muscorum was obtained from the Kaiser Research Foundation and was made bacteria free by Dr. J. A. Lauritis. Modified Chu 10 culture medium (Chu, 1942) was used throughout the experiment. Bactotryptone (Difco. Lab., 0.3 mg/l of the culture medium) was used as a nitrogen source instead of soil extract.

One hundred ml of cell suspension was used as inoculum for every liter of culture medium in a 2,800 ml Fernbach flask. All cultures were maintained on a 12-hour alternating light and dark cycle. The cultures were illuminated by 100 foot-candle light during the first 6 days and then were transferred to 20 foot-candle light condition for another 6 days, all at 23°C. Cultured cells were harvested by centrifugation at 5,000xg for 10 minutes. Harvested cells were washed in either potassium phosphate buffer (0.1 M, pH 7.4) or tris-HCl buffer (10 mM tris(hydroxymethyl) aminomethane, 10 mM magnesium acetate, pH 7.4) and pelleted at the same speed. The pelleted cells were immediately frozen in liquid nitrogen and stored at -20°C before use.

Extraction and Purification

Forty grams (wet weight) of algal cells were suspended in 100 ml cold phosphate buffer (0.1 M, pH 7.4) or tris-HCl buffer (10 mM tris, 10 mM magnesium acetate, pH 7.4) and the suspension was passed through a liquid nitrogen cooled Ribi-fractionator at 20,000 psi. The resulting

extract was centrifuged for 10 minutes at 2,000xg to eliminate unbroken cells and large fragments. Sodium deoxycholate was added to the supernatant to make a 1% final concentration, and was allowed to stand at 4°C for several hours with occasional stirring. Large particles were removed by centrifugation at 5,000xg for 10 minutes and supernatant was stored at -20°C.

Alpha-granule purification

Alpha-granules were pelleted by 10 minutes centrifugation of the crude extract in potassium phosphate buffer (0.1 M, pH 7.4) at 15,000xg. Centrifugation of resuspended material at low speed (5,000xg for 10 minutes) and high speed (15,000xg for 10 minutes) eliminated gross contamination. The crude α-granules obtained by this method may be used immediately or stored at -20°C.

Further purification was achieved by sucrose density gradient centrifugation. Two ml of the crude α-granule were layered on top of a linear sucrose gradient tube (10 to 30% sucrose). Three gradient tubes of the same preparation were centrifuged for 4 hours at 23,000xg in a Spinco SW 25.1 swinging bucket rotor using a Spinco L-4 preparative ultracentrifuge. Twenty-four fractions of 2 ml each were collected at the end of the run by either puncturing the bottom of the tube or forcing the gradient to move upward through a small tube by a mechanical pump. Each fraction was negatively stained and examined under an electron microscope. Fractions containing α-granules were pooled together and sucrose was removed by centrifugation at 20,000xg.

Ribosome purification

Cell homogenate in tris-HCl buffer was adjusted to 5 mM mercaptoethanol and 1% Bentonite and then centrifuged for 10 minutes at 25,000xg to remove large particles. Ribosomes were recovered by centrifugation of the supernatant for one hour in a No. 50 rotor at 100,000xg using a Spinco L-4 preparative ultracentrifuge. The pellet was washed twice in the same buffer without Bentonite and crude ribosomes were pelleted at the same speed. Purification procedures were carried out in a cold room and rotor temperature was always kept at 4°C.

Further purification of ribosomes was again achieved by sucrose density gradient centrifugation. The procedures were similar to that of α-granule purification except tris-HCl buffer (10 mM tris-HCl, 10 mM magnesium acetate and 5 mM mercaptoethanol, pH 7.4) was used instead of potassium phosphate buffer. Ribosome-containing fractions were identified by measuring the content of each tube at 260 nm using a Beckman model DB-G spectrophotometer. Sucrose was finally removed by centrifugation at 100,000xg.

Alpha-Granule Studies

Electron microscopy

Cells were collected from vigorously growing cultures by centrifugation for 10 minutes at 5,000xg. Pelleted cells were embedded in 2% agar, chilled, and small blocks were fixed in phosphate-buffered 3% glutaraldehyde (Sabatini et al., 1963). After post-fixation in 1% osmium tetroxide, blocks were dehydrated in an ethanol series and embedded in

Epon 812. Sections were stained with uranyl acetate and lead citrate.

For negative staining, a dilute suspension of α-granules in potassium phosphate buffer (0.1M, pH 7.4) was mixed with an equal volume of 2% potassium phosphotungstate (adjusted to pH 7.4 with potassium hydroxide) and applied to formvar-coated grids by spraying or using a platinum loop (Brenner and Horne, 1959).

Both thin sections and negatively-stained preparations were examined with a Hitachi HU-11C electron microscope operated at 50 or 75 kv. The specimen stage was equipped with an anticontamination device cooled by liquid nitrogen.

Analytical centrifugation

Sedimentation coefficient analyses were carried out in a Spinco model E analytical ultracentrifuge. All runs were made at 20°C and sedimentation patterns were recorded with Schlieren optics. Concentrations of α-granules ranging from 3 to 8 mg/ml in potassium phosphate buffer (0.1 M, pH 7.4) were used for extrapolation to infinite dilution. Both viscosity and density were corrected to that of water at 20°C.

Chemical analysis

Alpha-granules were hydrolyzed by using either hydrochloric acid or α-amylase free-β-amylase (Calbiochem.). Paper chromatographic separation of sugars was according to Putman's method (1957). Total glucose was determined by Nelson's method (1944).

Colorimetric determination of the glycogen-iodine complex was according to Krisman's procedure (1962). Both qualitative and quantitative measurements were carried out in saturated calcium chloride solution. The

absorption spectrum of the iodine-glycogen complex was compared with those of potato starch (J. T. Baker Chemical Co.), rabbit liver glycogen (Mann Research Lab.) and Shell-fish glycogen (Sigma Chemica' Co.). A Beckman model DB-G spectrophotometer equipped with a logarithemic recorder was used for spectra determinations.

The average chain length of Nostoc glycogen was determined by the periodate oxidation method (Hassid and Abraham, 1957). Complete oxidation of glycogen was checked against a sucrose blank.

One mg/ml α-amylase (Calbiochem) in potassium phosphate buffer (0.1M, pH 6.5) was used for α-amylolysis. The reaction was carried out in a 37°C water bath until no further increase in glucose was detected. Alpha-macrodextrin was precipitated by 70% ethanol, and analyzed for glucose content.

Protein content was determined by Lowry's method (Lowry et al., 1951) using bovine serum albumin as a standard.

Ribosome Studies

Determination of Nostoc ribosome spectrum and composition

The ultraviolet absorption spectrum of purified Nostoc ribosomes in tris-HCl buffer (1 mM tris-HCl, 5×10^{-1} mM magnesium acetate, pH 7.4) was determined using a Beckman model DB-G spectrophotometer. Samples to be analyzed for RNA and protein were acidified with cold trichloroacetic acid, to a final concentration of 7%. Precipitates were collected after an hour incubation at 4°C and washed several times with cold 7% trichloroacetic acid. The nucleic acid was extracted from the precipitate with 7% trichloroacetic acid at 95-99°C for 10 minutes and the concentration of

RNA in the extract was estimated by the orcinal method of Mejbaum (1939) with yeast soluble RNA as a standard. The hot trichloroacetic acid insoluble pellet was dissolved in 0.2 N sodium hydroxide and the concentration of protein was determined by Lowry's method (Lowry et al., 1951) with bovine serum albumin as a standard.

Determination of ribosomal RNA base composition

Preparation of ribosomal RNA Ribosomal RNA was extracted with phenol according to the procedure of Rifkin (Rifkin et al., 1967). Purified ribosomes were suspended in equal volumes of tris-HCl (10 mM, pH 7.4) and water-saturated phenol (analytical grade from Fisher Scientific Co.). The mixture was stirred with a magnetic stirrer for 45 minutes at 4°C. At the end of the extraction period, the mixture was centrifuged at 15,000xg for 20 minutes. The aqueous layer was carefully removed and adjusted to a concentration of 0.1 M sodium chloride and 10 mM sodium acetate (pH 5.1). RNA was precipitated by addition of 2.5 volumes of absolute ethanol. Traces of phenol were removed by repeated precipitation with ethanol. Any material that did not redissolve after ethanol precipitation was removed by centrifugation.

Base composition determination Ribosomal RNA was hydrolyzed by dissolving the RNA in 0.3 N potassium hydroxide at a concentration of about 0.5 mg/ml and incubated for 18 hours at 37°C (Rifkin et al., 1967). After hydrolysis the solution was acidified with 0.25 M perchloric acid. Trace amounts of precipitate were removed by centrifugation and the supernatant was adjusted to pH 7.5 with potassium hydroxide.

Base composition was determined by column chromatography (Hurlbert

et al., 1954). Three milligrams of hydrolyzed RNA was applied to a 20x1.5 cm Dowex-1 formate column (200-400 mesh) and nucleotides were eluted by ammonium formate salt gradient at relatively constant pH (pH 5.0-4.0). The gradient was started with distilled water and approched 1 M ammonium formate at the end of the run. One hundred fractions of 5 ml each were collected and nucleotide-containing fractions were identified by measuring absorbance at 260 nm with a Beckman DB-G spectrophotometer. Base ratios were calculated from absorbance measurements of each fraction in 0.1 M sodium phosphate buffer (pH 7.0). The amounts in micromoles of each nucleotide were calculated from the readings at 260 nm using the following extinction coefficients: 12.7 for cytidylic acid, 14.4 for adenylic acid, 9.9 for uridylic acid and 12.2 for guanylic acid (Rifkin et al., 1967).

Analytical centrifugation

Sedimentation analyses were carried out in a Spinco model E analytical ultracentrifuge using an An-D rotor. All runs were made at 10 to 12°C and sedimentation patterns were recorded with Schlieren optics. Concentrations ranging from 1 to 3 mg/ml of ribosomes in 10 mM tris-HCl buffer (pH 7.4) were used for extrapolation to infinite dilution. The relative amount of each component was estimated by measuring the area under each boundary. The sedimentation coefficients were corrected to the viscosity and density of water at 20°C. Magnesium acetate concentrations ranging from 1 to 10 mM were used for dissociation studies.

Amino acid incorporation

In vitro amino acid incorporation was carried out using 0.2 mg of ribosomes suspended in an incubation mixture (Nirenberg and Matthaei, 1961).

each incubation mixture contained 10 mM tris-HCl (pH 7.4), 10 mM magnesium acetate, 6 mM mercaptoethanol, 0.4 mg pH 5 enzyme fraction from Nostoc cells, 50 µg creatine phosphokinase (Calbiochem), 30 µg creatine phosphate (Calbiochem), 1 mM GTP (Nutritional Biochemical Co.), 20 µM ATP (Calbiochem), and 5 µM each of 19 unlabeled amino acids plus 5 µM of C^{14}-leucine (0.5 µc specific activity, Calbiochem). Concentrations of cycloheximide (Calbiochem) ranging from 50 µg/ml to 500 µg/ml and chloramphenicol (Calbiochem) ranging from 10 µg/ml to 200 µg/ml were added separately into the incubation mixtures to test the drug sensitivities of the Nostoc amino acid incorporating system. After incubation at 37°C for 45 minutes reactions were stopped by adding 2 ml of cold 5% trichloroacetic acid. The contents of each tube were mixed and kept at 0 to 4°C. After 24 hours the tubes were heated at 90°C for 20 minutes with gentle agitation and were then cooled to room temperature. One milliliter of water was added to each vial and the mixture was shaked for 15 minutes in a shaker. Ten milliliter of counting fluid (100 gm naphthalene and 60 gm 2,5-diphenyloxazole in one liter of p-dioxine) was then added to each vial and shaked again for 30 minutes at room temperature. Radioactivity was counted in a Beckman DPM-100 scintillation counter. A sample containing all reagents but without antibiotics was treated with trichloroacetic acid prior to incubation and used as a control.

RESULTS

Characterization of Alpha-Granules

Morphology and size distribution

Nostoc α-granules are located between the photosynthetic thylakoids (Figure 1). These granules are generally uniform in size and shape. Most profiles in sections are spherical. Occasionally elongated granules are seen. The observed variations apparently resulted from sectioning in different planes.

Purified α-granules are uniform in size and shape (Figure 2). Each individual particle has a bipartate appearance. The granules are stable to changes in pH from 3.5 to 8.0. Many small subunits are observed in each granule at high magnification (Figure 3). These subunits are similar to the γ-structures of animal glycogen (Drochmans, 1962). They appear spherical or occasionally elongate with diameters of 2 to 2.5 nm and lengths up to 6 nm.

For size measurement, randomly selected electron micrographs were enlarged to 200,000 times and 800 α-granules were measured for width and 950 α-granules were measured for length. The variations in width and length are presented here in histograms (Figures 4 and 5). These particles average 31 nm in width and 65 nm in length. Occasionally particles are observed that are much shorter or longer. Alpha-granules partially degraded by α-amylase appear to be flattened and polydispersive in size (Figure 6).

Ethanol precipitation is commonly used to prepare animal glycogen.

This simple method was also used for α-granule purification instead of sucrose density gradient separation after their glycogen-like properties were identified. Thoroughly washed α-granules were precipitated with 70% ethanol repeatedly and purity was checked by both chemical analysis and negative staining. Precipitated α-granules are contaminated with a high percentage of proteins, sometimes reaching 8 to 10%. It is clear that many proteins are co-precipitated with α-granules in 70% ethanol and can not be removed by mild procedures. Removal of proteins by concentrated potassium hydroxide treatment was considered too destructive and was not used. In addition, repeated ethanol precipitation also caused α-granules to disintegrate. Alpha-granules of irregular shape and small particles, possibly resulting from α-granule breakdown, are frequently observed in ethanol-precipitated preparations (Figure 7). Density gradient separation rather than ethanol precipitation is recommended for the purification of α-granules as well as other glycogens for physical and chemical studies.

Sedimentation studies

Nostoc α-granules sediment as a single component in the ultracentrifuge (Figure 8). The sedimentation coefficient varied from 220S to 245S depending on solute concentrations. A value of 265S was obtained after extrapolation to infinite dilution (Figure 10).

Chemical analysis

The protein content of *Nostoc* α-granule preparations decreased at a constant rate with degree of purification, down to about 2 to 3% protein after thorough washing.

Only glucose was identified from acid-hydrolyzed *Nostoc* α-granules

Table 1. Relationships between culture age and branching characteristics of Nostoc glycogen[a]

Age of culture in days	Average chain length	Degree of[b] branching	Percent yield of α-macrodextrin
10	10.9	9.2	11.0
12	13.2	7.6	12.8
14	13.6	7.4	12.9
20	13.1	7.6	13.3

[a]Average value of 4 determinations

[b]Reciprocal of average chain length x 100

by paper chromatography. Maltose was identified by the same method after α-amylase-free β-amylase digestion of the Nostoc α-granules. The yield of glucose from acid hydrolysis accounted for more than 95% of the α-granule weight. The absorption spectrum of α-granule-iodine complex had its λ_{max} at 410 nm. When compared to several well-characterized glycogens the absorption spectrum of the complex was most similar to that of shell-fish glycogen (Figure 12). Nostoc glycogen has an average chain length of 13 glucosyl units as determined by periodate oxidation. In another experiment native α-granules were degraded by α-amylase and the resulting α-macrodextrin was determined. The 11 to 13% yield of α-macrodextrin suggests that the degree of branching is high (Table 1). Both average chain length and α-macrodextrin yield of Nostoc glycogen appear to vary slightly with the culture age (Table 1).

Characterization of Ribosomes

UV spectrum and composition of Nostoc ribosomes

The ultraviolet absorption spectrum of Nostoc ribosomes was determined in 1 mM tris-HCl, $5x10^{-1}$ mM magnesium acetate (pH 7.4) solution (Figure 13). The absorbance ratio of 260/280 is 1.9 and that of 260/240 is 1.6. This result indicates a low cytidylic acid content in the ribosomal RNA as in most light type (70S) ribosomes. Similar results were reported for Euglena gracilis chloroplast ribosomes (Eisenstadt and Brawerman, 1964) and Escherichia coli ribosomes (Tissieres et al., 1959).

Purified Nostoc ribosomes comprise 63±2% ribosomal RNA as determined by the orcinal method and 38±1% ribosomal protein as determined by Lowry's method. The RNA to protein ratio is high in Nostoc ribosomes compared to that reported for heavy type (80S) ribosomes (T'so et al., 1958). This high RNA to protein ratio is typical in both chloroplast (Eisenstadt and Brawerman, 1964) and bacterial (Tissieres et al., 1959) ribosomes.

Since Nostoc ribosomal RNA composition (Table 2) is close to that of Escherichia coli, the value $E_{260}^{0.1\%}=25$ is used as extinction coefficient for Nostoc ribosomal RNA (Spahr and Tissieres, 1959). The extinction coefficient of Nostoc ribosomes was estimated as $E_{260}^{0.1\%}=15.7$ using 25 as the ribosomal RNA extinction coefficient and the previously determined RNA to protein ratio of Nostoc ribosomes. The average value of extinction coefficient of Escherichia coli ribosomes was reported as 16 (Tissieres et al., 1959).

Table 2. Nucleotide composition of Nostoc ribosomal RNA

Nucleotides	Moles percent[a]
Cytidylic acid	18.3 ± 0.3%
Uridylic acid	22.8 ± 0.5%
Adenylic acid	26.8 ± 0.6%
Guanylic acid	32.3 ± 0.5%

[a] Average values of four determinations

Base composition of Nostoc ribosomal RNA

The base composition of Nostoc ribosomal RNA determined by column chromatography is given in table 2. A small amount of minor nucleotides was resolved by column elution but was not identified. The composition of the four major nucleotides is similar to that of Escherichia coli (Spahr and Tissieres, 1959) and chloroplast (Eisenstadt and Brawerman, 1964) ribosomal RNA nucleotides. The low content of cytidylic acid is typical of the light type (70S) ribosomal RNA and is also reflected on its ultraviolet absorption spectrum (Figure 13).

Sedimentation properties of Nostoc ribosomes

The sedimentation patterns of Nostoc ribosomes were influenced by magnesium ion concentrations. The predominant component was ribosome monomers at magnesium ion concentration of 5 mM of higher (Figure 9a). A small amount of aggregated dimers was also present at this magnesium ion concentration. The monomers were dissociated into two subunits when

magnesium ion concentration was reduced to 3 mM and lower by dialysis (Figure 9b). Sedimentation coefficients were extrapolated to infinite dilution and 69 ± 1S, 48 ± 2S and 32 ± 2S were obtained for the monomer, large subunit and small subunit repectively (Figure 11). Data obtained from least square analysis gave 68.5S for the monomer, 48.1S for the large subunit and 31.4 for the small subunit.

Amino acid Incorporation

Purified ribosomes as well as precipitated pH 5 enzymes were used to investigate the in vitro amino acid incorporating system of Nostoc muscorum. C^{14}-leucine incorporation in this system is sensitive to chloramphenicol, a drug which also inhibits peptide synthesis in bacteria. The dosage of chloramphenicol required for 90% protein synthesis inhibition is between 50 to 100 ug/ml (Table 3). Cycloheximide, a peptide synthesis inhibitor of almost all eucaryotic cells, had no effect on the protein incorporating system of Nostoc, even at a dosage of 1,500 ug/ml. This result again suggests the similarities between blue-green algae and chloroplast (Hoober and Siekevitz, 1968).

Table 3. Effects of chloramphenicol and cycloheximide on the amino acid incorporating system of Nostoc muscorum

Treatment	Concentration in μg/ml	CPM[a]
Complete system		1885
Control[b]		186
Complete system plus chloramphenicol	20	1892
	30	2224
	50	1810
	100	314
	200	247
Complete system plus cycloheximide	100	1433
	200	1873
	700	1394
	1000	1688
	1500	1266

[a]Average values of four runs

[b]Protein precipitated prior to incubation

DISCUSSION

It has been suggested that spherical glycogen granules are formed by the branching of polyglucosyl chains at more or less regular intervals (Pollard, 1957). As the size of the molecule increases, there comes a point at which the periphery is so densely packed by the non-reducing ends of the branches that no more glucose units can be packed into the sphere. The maximum theoretical diameter of such a sphere is between 26 to 40 nanometers. This model agrees with the size of many extensively studied glycogen particles. For example, the diameter of native glycogen particles range from 35 to 40 nm (214S) in Tetrahymena (Barber et al., 1965), 40 nm (115-135S) in rabbit muscle (Wanson and Drochmans, 1968), to 48 nm (220-230S) in rat liver (Drochmans and Dantan, 1968). Phytoglycogen, only found in sweet corn, has a diameter of 35 nm and a sedimentation coefficient of 200S (Madsen and Cori, 1958). Compared with glycogen from other sources, Nostoc glycogen has a higher sedimentation coefficient (265S) and has a unique elongate rather than spherical shape (30 x 65 nm with a central constriction). Since the intact granule can not be readily dissociated into spherical subunits, it appears that either a unique " backbone " or a different type of branching is involved in this type of glycogen. Giesy's description of polyglucosides granules in Oscillatoria chalbea (Giesy, 1964) where the diameter of each granule is about 35 nm but where great lengths are often seen, supports this conclusion.

Nostoc glycogen is chemically similar to most animal glycogens, of special interest is the 11 to 13% yield of α-macrodextrin upon α-amylolysis

of Nostoc glycogen. The reported values for α-macrodextrin yield upon α-amylolysis were 11.7% for shell-fish glycogen, 2.4% for rabbit liver glycogen, 1.1% for phytoglycogen and 0.4% for amylopectin (Heller and Schramm, 1964). It is known that pure α-amylase can not cleave α—1→4 linkage when the linkage is in the close vicinity of the α—1→6 linkage (Roberts and Whelan, 1960). The high yield of α-macrodextrin therefore indicate a high degree of branching of the native polymer. The λ_{max} of glycogen-iodine complex is considered to vary with the external chain length (Wanson and Drochmans, 1968). The iodine complex of rabbit muscle glycogen (Average external chain length of 16 to 18 glucosyl units) has a λ_{max} at 515 nm and that of mammalian liver (Average external chain length of 8 to 10 glucosyl units) has a λ_{max} at 460 nm. Nostoc glycogen-iodine complex, similar to that of shell-fish glycogen, has a λ_{max} at 410 nm suggesting short external chain lengths. Further biochemical studies are required to elucidate the properties of Nostoc glycogen in detail.

Early studies indicated that glycogens are present in bacteria (Chargaff and Moore, 1944; Dagley and Dawes, 1949; Hehre and Hamilton, 1952; Barry et al., 1952). Recently Antoine and Tepper (1969) reported that mycobacterial glycogens are variable in size and have no clear sub-unit structure. The chemical properties, such as short external chain length and high degree of branching, are similar to those of Nostoc glycogen.

The results of this study indicate that the proposed name poly-glucoside granule (Giesy, 1964; Lang, 1969) is an acceptable one, however, a more accurate name would be glycogen granule.

Since the discovery of chloroplast DNA (Stocking and Gifford, 1959), evidence has accumulated in support of the old notion that chloroplasts are somewhat independent of nuclear control. Spencer and Whitfield (1969) reported that chloroplast DNA polymerase is different from that of the nucleus. Similar results were reported on chloroplast DNA-dependent RNA polymerase (Tewari and Wildman, 1969). Rifampin, a bacterial DNA-dependent RNA polymerase inhibitor, was also found to inhibit chloroplast DNA-dependent RNA polymerase (Surzycki, 1969). Ranelletti et al. (1969) further demonstrated that isolated chloroplasts were able to incorporate free amino acids into proteins. The results obtained on Nostoc ribosomes suggest that ribosomes from bacteria, blue-green algae, chloroplast and possibly mitochondria are closely related. Ribosomes from these organisms and organelles all belong to the 70S type. Their base composition is similar and cytidylic acid content is always lower that that of 80S type ribosomes (Rifkin et al., 1967; Spahr and Tissieres, 1959; Eisenstadt and Brawerman, 1964). Similar to ribosomes of blue-green algae, bacterial and chloroplast ribosomes have been shown to be sensitive to chloramphenicol (Nirenberg and Matthaei, 1961; Nathans and Lipmann, 1961; Hoober and Siekevitz, 1968). No reports have been noted with regard to the chloramphenicol sensitivity of mitochondrial ribosomes.

In addition to these physiological properties, the morphological similarity between chloroplasts and blue-green algae is striking as is the similarity in the photosynthetic mechanisms of these two groups. This all supports the hypothesis forwarded by Mereschkowski (1905) and others who propose a symbiotic origin of chloroplasts. On the other hand, the presence of glycogen in blue-green algae argues against their possible

role in such an origin. However, it is known that there are several classes of enzymes involved in homopolysacchride synthesis. One class of enzymes, the synthetases, is responsible for glucosyl chain elongation. The other class, the branching enzyme, is responsible for branching properties (Larner, 1953). The particulate polysacchride formed is the net result of the action of both classes of enzymes. It is not unlikely that in the course of evolution the synthetases remained essentially the same in both chloroplasts and blue-green algae, while the branching enzymes evolved along different paths in chloroplasts and blue-green algae over the years. Alternatively, the original branching enzyme of primitive chloroplasts may have been lost and the present day branching enzyme in chloroplasts may be specified by the nucleus. Hoober and Siekevitz (1968) reported that chloroplast and cytoplasmic protein synthesizing systems control membrane and chlorophyll synthesis in chloroplasts. Apparently chloroplasts synthesize only part of their constituents and the other constituents, including some enzymes, come from the nucleus via the cytoplasm. Certain differences between chloroplasts and blue-green algae are therefore expected since protein synthesizing systems in plastids are free to mutate and thus be lost, if there are nuclear-cytoplasmic systems that could substitute. Further studies, especially DNA-RNA hybridization study, are required to elucidate their relationships in detail.

Summary

1. Alpha-granules and ribosomes were isolated from a blue-green alga, Nostoc muscorum, in large quantities and high purity for physical and chemical studies.

2. Purified α-granules have average dimension of 31 nm in width and 65 nm in length with a central constriction. The sedimentation coefficient of purified α-granules is 265S.

3. Chemical analysis reveals that α-granules contain highly branched polyglucosyl units with short external chain length.

4. Purified ribosome monomers sediment at 68.5S. At magnesium ion concentration of 3 mM or lower the monomers dissociate into two subunits of 48.1S and 31.4S.

5. Purified ribosomes comprise 63±2% ribosomal RNA and 38±1% ribosomal protein. The ribosomal RNA consists of 18.3±0.3% cytidylic acid, 22.8±0.5% uridylic acid, 26.8±0.6% adenylic acid and 32.3±0.5% guanylic acid.

6. Nostoc ribosomes are sensitive to chloramphenical but insensitive to cycloheximide. The in vitro amino acid incorporation of Nostoc ribosomes was reduced to 10% of normal incorporation by 100 μg/ml of chloramphenicol.

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APPENDIX

Figure 1. Alpha-granules are located between photosynthetic thylakoids (arrows) as shown in a sectioned Nostoc muscorum cell. Fixed in glutaraldehyde and osmium. Stained with uranyl acetate and lead citrate. 50,000 X


200 nm
1

Figure 2. Negatively stained α-granules. 100,000 X

Figure 3. Negatively stained α-granules showing Y-structures (arrows). 400,000 X

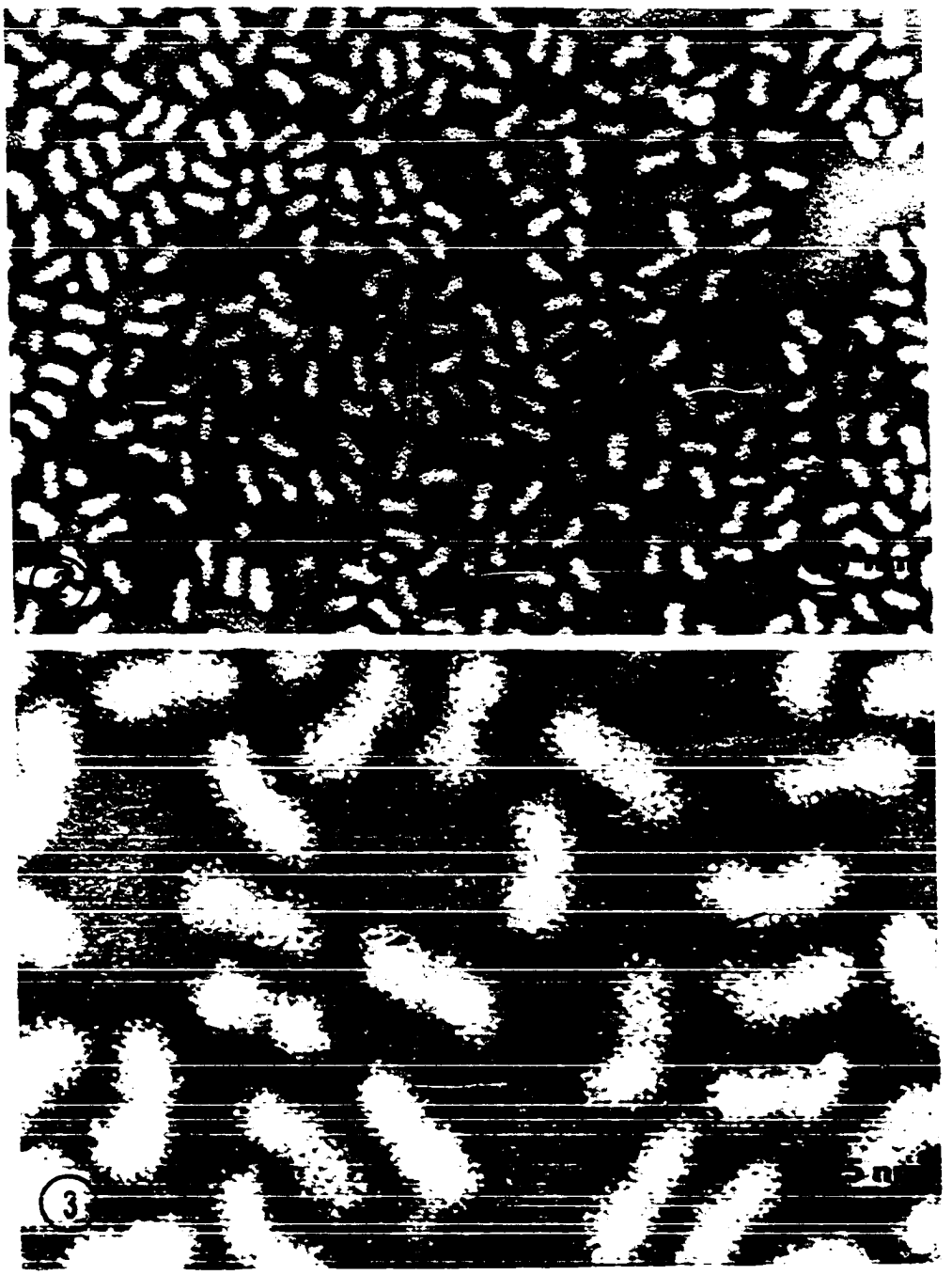

3

Figure 4. Width-frequency histogram of α-granules

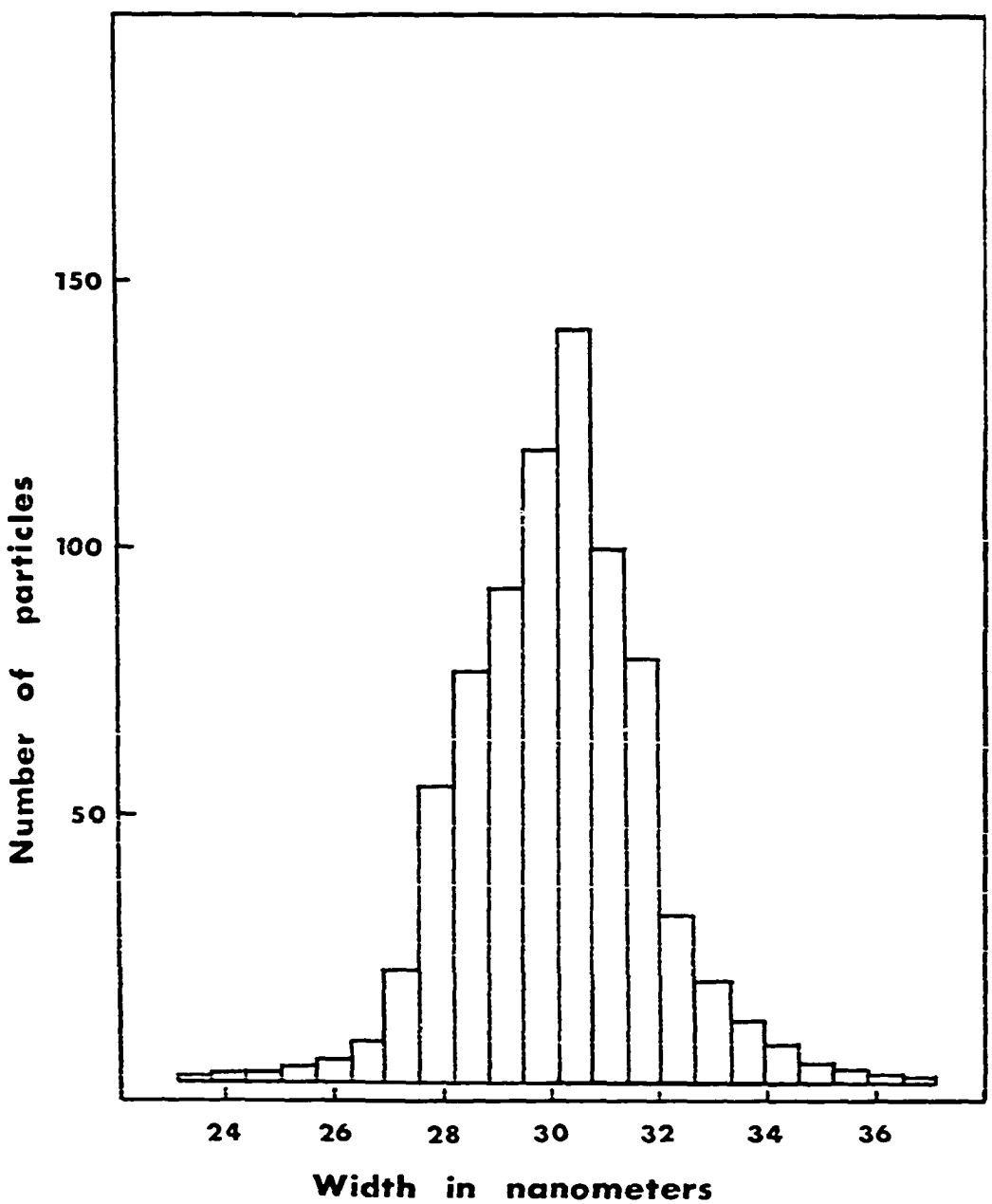

150
100
50
Number of particles
24
26
28
30
32
34
36
Width in nanometers

Figure 5. Length-frequency histogram of α-granules

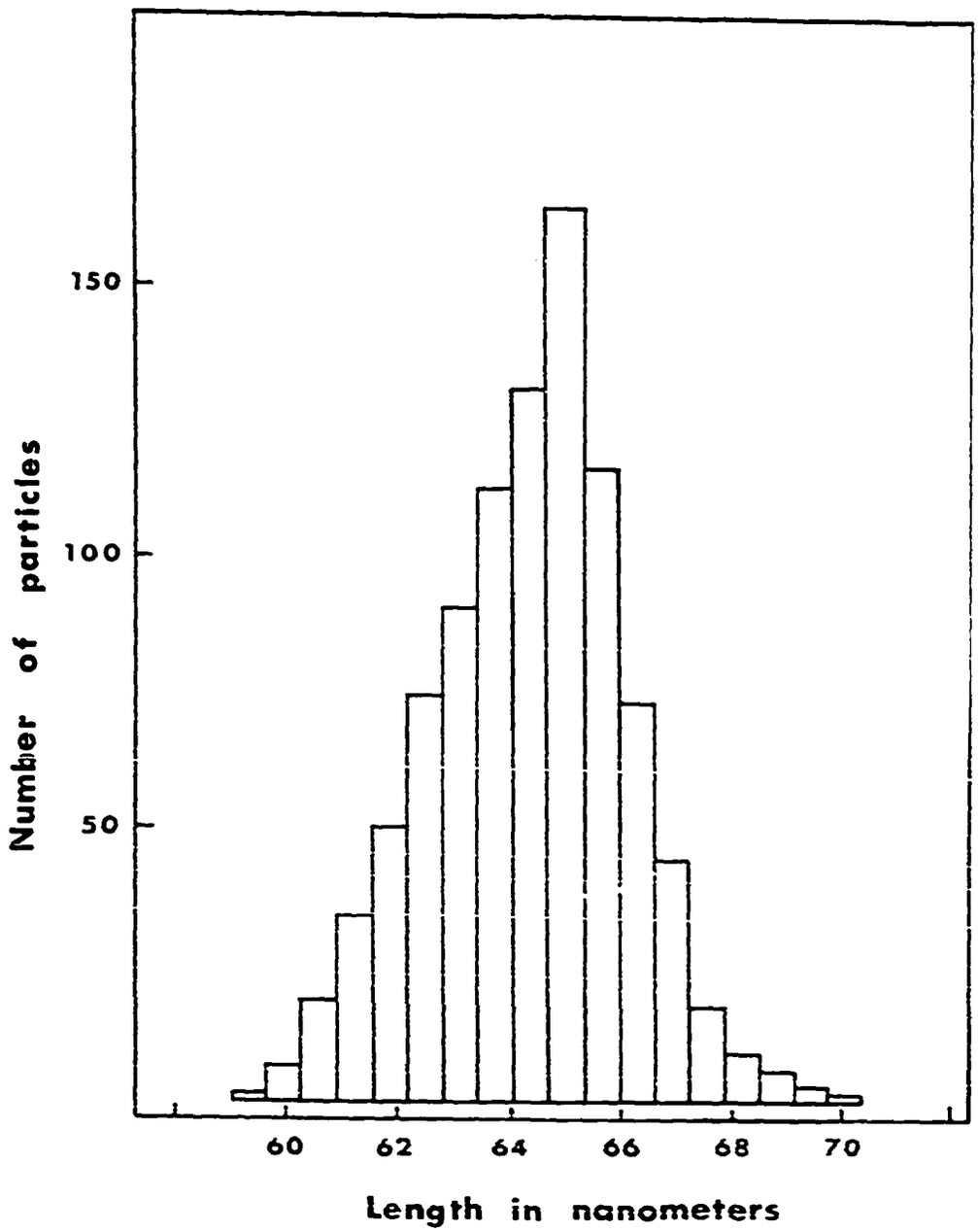

150
100
50
Number of particles
60
62
64
66
68
70
Length in nanometers

Figure 6. Alpha-amylase partially degraded α-granules. Negatively stained with potassium phosphotungstate. 100,000 X

Figure 7. Alpha-granules prepared by repeated ethanol precipitation. Negatively stained with potassium phosphotungstate. 100,000 X

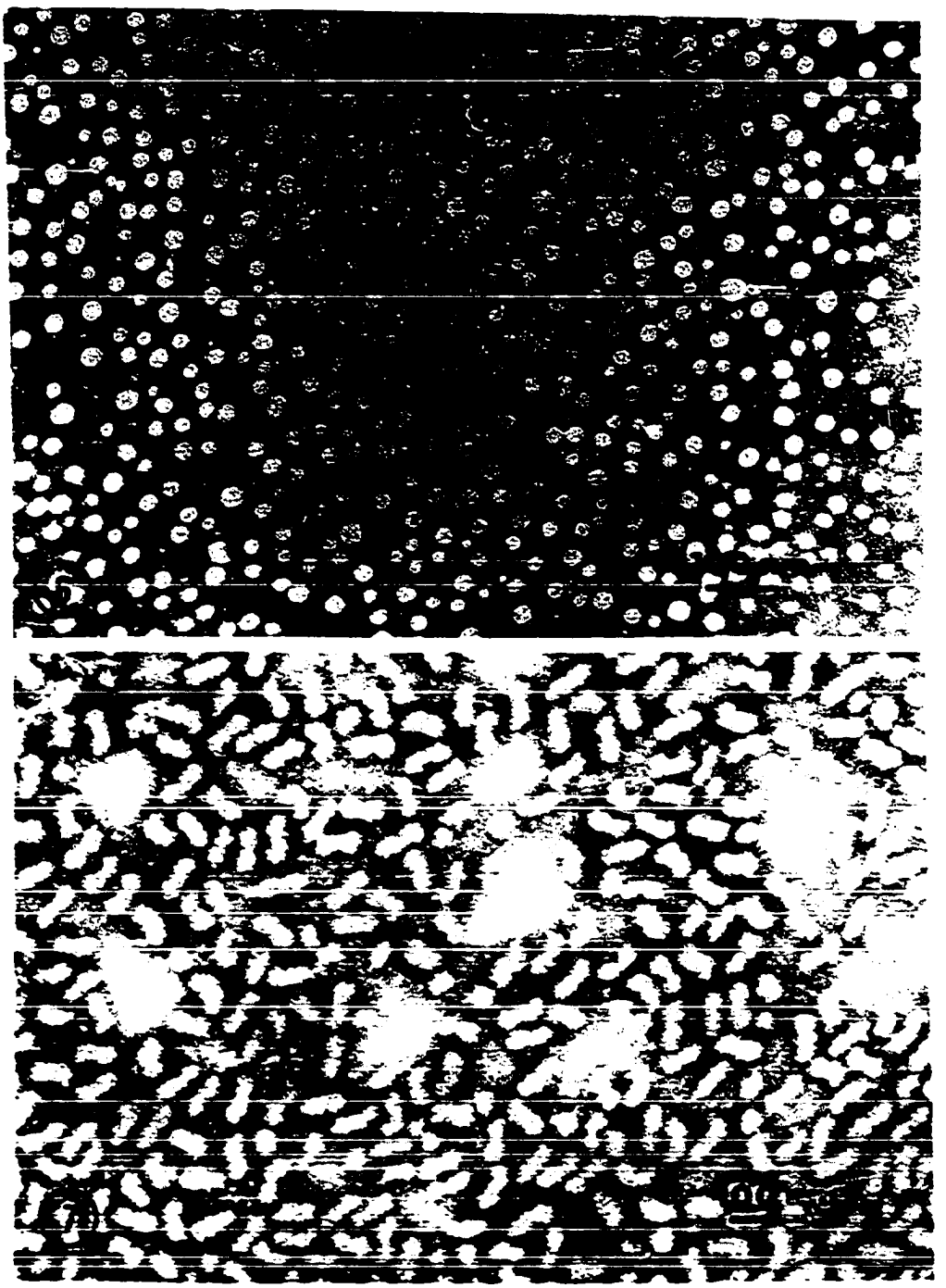
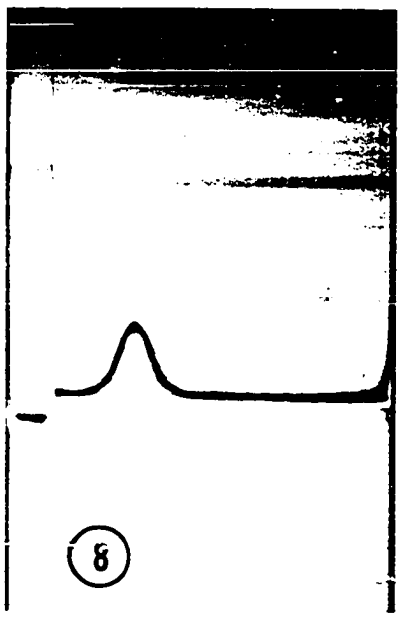


Figure 8. Analytical centrifugation pattern of purified α-granules in potassium phosphate buffer (0.1 M, pH 7.4). The photograph was taken 8 minutes after reaching 18,000 rpm. Sedimentation is from left to right

Figure 9. Analytical centrifugation patterns of Nostoc ribosomes in:
(a) 10 mM tris-HCl, 10 mM magnesium acetate, pH 7.4
(b) 10 mM tris-HCl, 1 mM magnesium acetate, ph 7.4
Photographs were taken (a) 3 minutes and (b) 6 minutes after reaching 52,000 rpm. Sedimentation is from left to right



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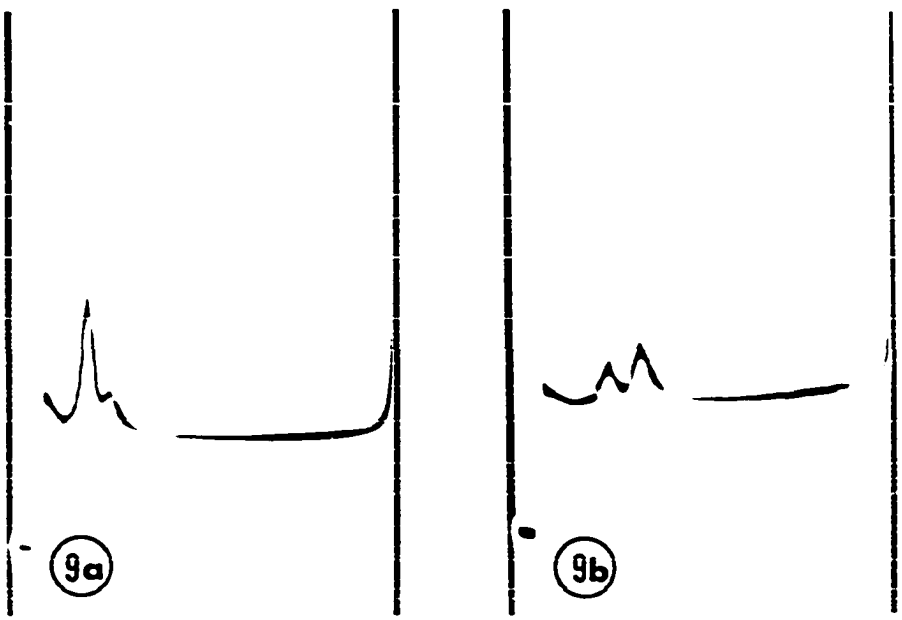
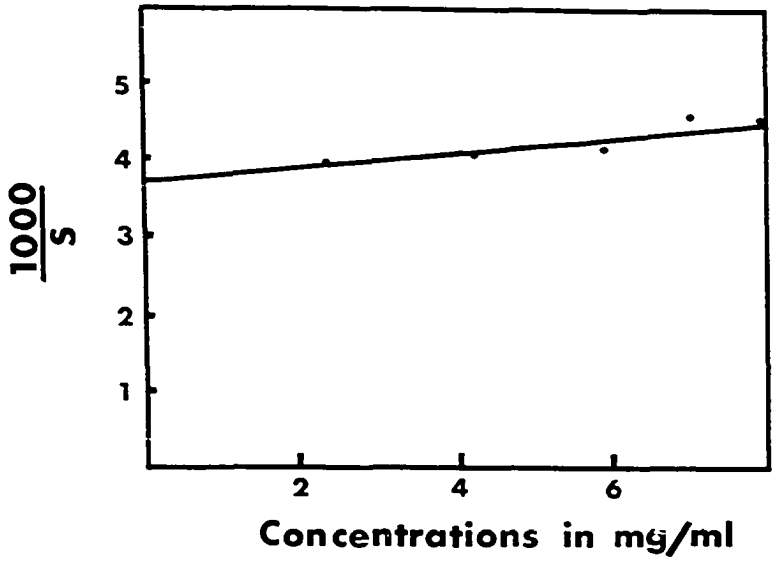


9a 9b

Figure 10. Sedimentation coefficient of α-granules extrapolated to infinite dilution

Figure 11. Sedimentation coefficients of Nostoc ribosomes and ribosomal subunits extrapolated to infinite dilution. Ribosomes (——), large subunits (←——→) and small subunits (- - -)


1000/S
5
4
3
2
1
2
4
6
Concentrations in mg/ml

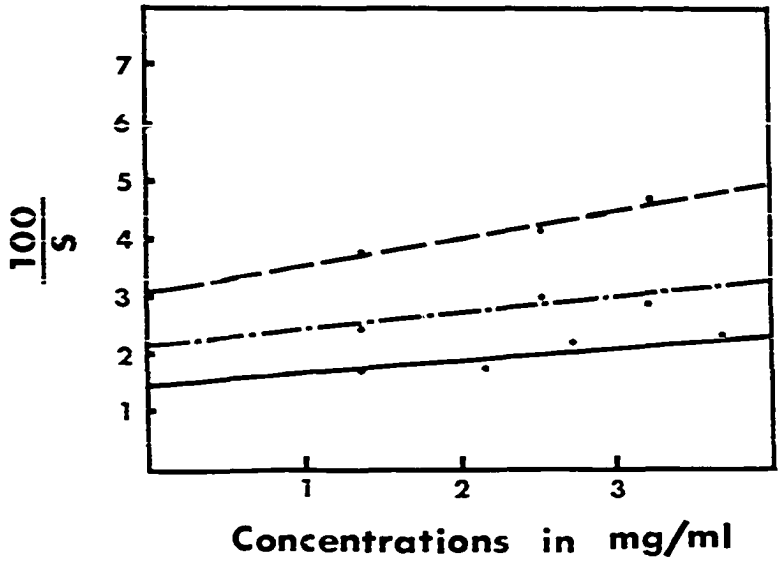

100/S
7
6
5
4
3
2
1
1
2
3
Concentrations in mg/ml

Figure 12. Spectra of iodine complex of Nostoc α-granules (———), shell-fish glycogen (------), rabbit liver glycogen (—·—·—) and potato starch (—•—•—). All in saturated calcium chloride solution

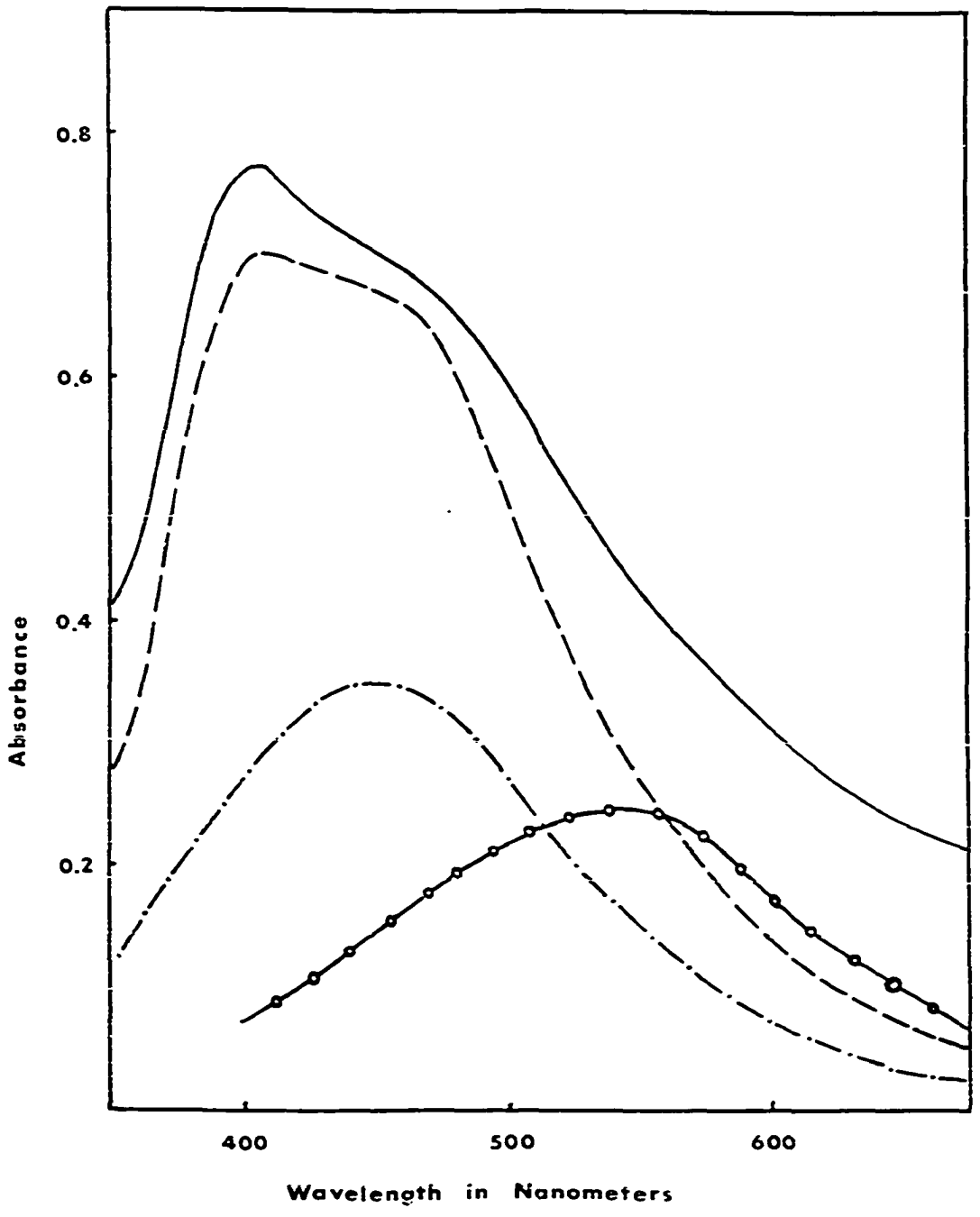
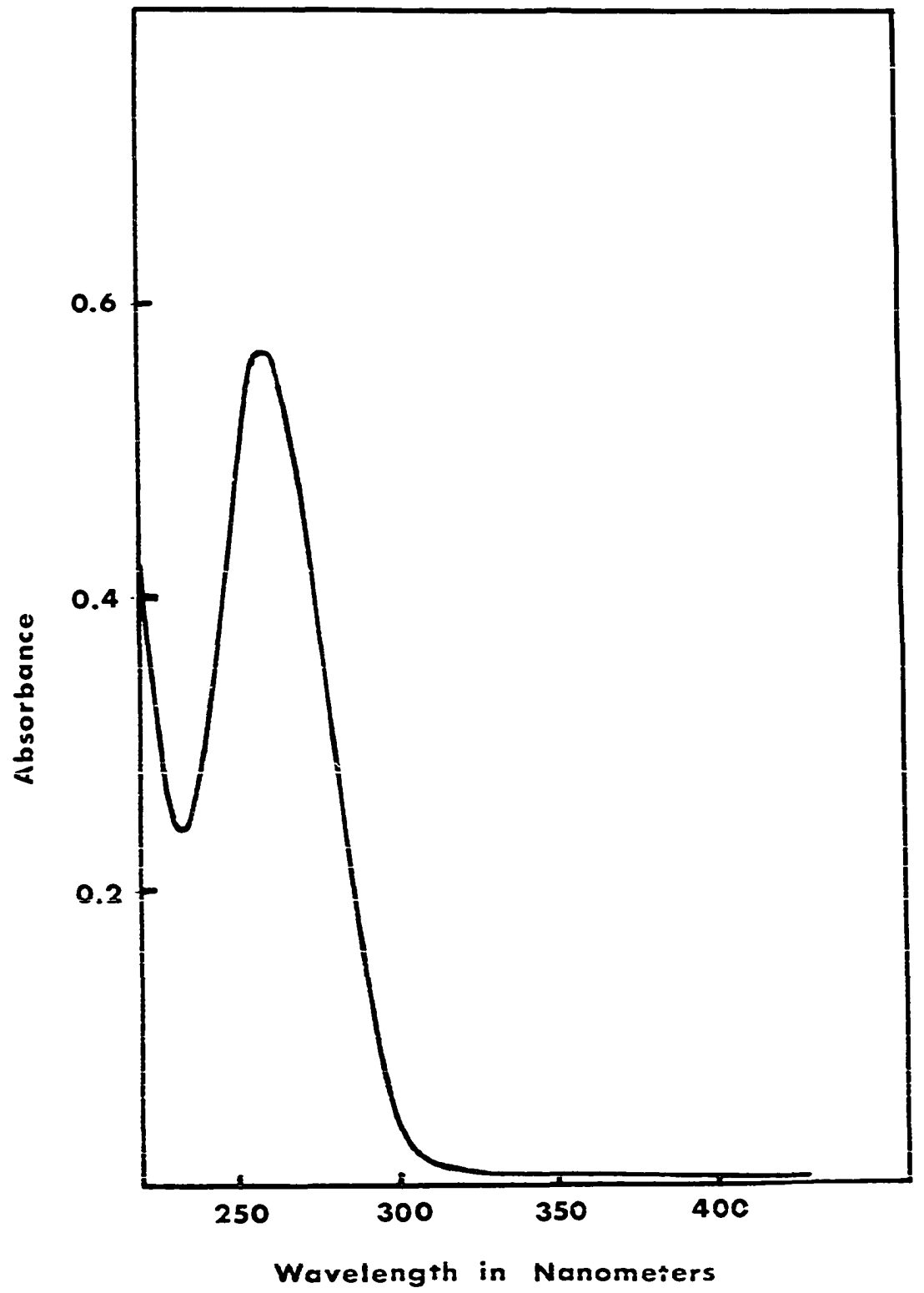

0.8
0.6
0.4
0.2
Absorbance
400
500
600
Wavelength in Nanometers

Figure 13. The UV absorption spectrum of Nostoc ribosomes in 1 mM tris-HCl, 5×10^{-1} mM magnesium acetate, pH 7.4


0.6
0.4
0.2
Absorbance
250
300
350
400
Wavelength in Nanometers